

The Crystal Structure of Biotin Synthase, an S-Adenosylmethionine-Dependent Radical Enzyme

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The crystal structure of biotin synthase addresses how “AdoMet radical” enzymes, also called “Radical SAM” enzymes, use an Fe₄S₄ cluster and S-adenosyl-L-methionine to generate organic radicals. Biotin synthase catalyzes the radical-mediated insertion of sulfur into dethiobiotin (DTB) to form biotin (vitamin B₈). The structure places the substrates, i.e. DTB and AdoMet, between the Fe₄S₄ cluster (essential for radical generation) and the Fe₂S₂ cluster (postulated to be the source of sulfur), with both clusters in unprecedented coordination environments.

Biotin is an essential vitamin that plays a ubiquitous role in human growth and metabolism. Biotin deficiency results in skin lesions, abnormal fat distribution, neurological symptoms, and immunodeficiency. A low biotin level has also been correlated to an increased incidence of type II diabetes mellitus. Biotin is a valuable commercial commodity, used as an additive in food, health, and cosmetic products, and as a research tool in the biochemical sciences.

We recently reported the crystal structure of *E. coli* Biotin Synthase (BioB), which belongs to the emerging “AdoMet radical” or “radical SAM” enzyme superfamily. The superfamily is characterized by the presence of a conserved CX₃CX₂C motif, (“C” is the amino acid cysteine and “X” is any amino acid), and the requirement of a 4Fe-4S cluster and S-adenosyl-L-methionine (AdoMet or SAM) to perform carbon-based radical chemistry. Enzymes that utilize radical chemistry are able to catalyze challenging and unusual reactions, but are difficult to work with because of their aberrant reactivity and oxygen sensitivity. We were able to grow crystals of BioB in an anaerobic chamber and, over a 17-month period, obtained crystals of high-enough quality to solve the structure of this fascinating protein (**Figure 1**).

BioB activates DTB, a biotin precursor, for sulfur (S) insertion between two non-activated carbon atoms, forming biotin (**Figure 2**). The molecular basis for this remarkable chemistry lies in a novel reactivity of AdoMet in the presence of unique Fe-S clusters. BioB is a homodimer (i.e. formed by two identical molecules), with one active site per protomer and no interactions between the two active sites.

Each protomer adopts a common protein structure known as the “TIM barrel,” and contains two Fe-S clusters, both of which have unprecedented coordination

environments. At the C-terminal end of the TIM barrel, a 4Fe-4S cluster is coordinated by the three cysteines of the CX₃CX₂C motif, and a unique Fe of this cluster is coordinated by the amino and carboxyl groups of AdoMet. Buried deep in the center of the TIM barrel, a 2Fe-2S cluster is coordinated by three cysteines and one arginine, each of which is absolutely conserved. DTB is



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bound between AdoMet and the 2Fe-2S cluster (**Figure 3**).

The coordination of the 4Fe-4S cluster by AdoMet ensures efficient electron transfer from the cluster to the cofactor. The donation of one electron by the 4Fe-4S cluster to AdoMet results in reductive cleavage of the carbon-sulfur bond, generating the high-energy 5'-deoxyadenosyl radical (Ado•), an intermediate that has historically been associated with adenosylcobalamin (AdoCbl)-dependent radical enzymes. Ado• removes hydrogen atoms from DTB, preparing it for sulfur insertion. The unique 2Fe-2S cluster is proposed to be the S donor in the BioB reaction, and the close linear arrangement of AdoMet, DTB, and the 2Fe-2S cluster supports this hypothesis. It is possible that the arginine ligand plays a role in the reaction mechanism, or helps stabilize the 2Fe-2S cluster after sulfur insertion.

The structures of BioB and diol dehydratase, an AdoCbl-dependent radical enzyme, show remarkable similarities. Both deploy the TIM barrel fold to bind their radical-generating cofactors. Moreover, the active sites of BioB and diol dehydratase show that the positions of the substrate, cofactors, essential metals, and secondary structural elements coincide. These structural similarities point to the evolutionary relationship between the AdoMet-dependent and AdoCbl-dependent radical enzymes.

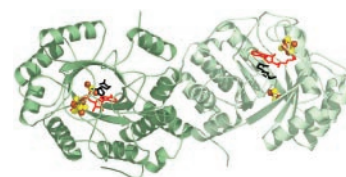


Figure 1. The BioB dimer. BioB is a homodimer, composed of two TIM barrel protomers (colored different shades of green). The 4Fe-4S cluster, which is present in all AdoMet radical proteins, is located at the C-terminal end of the cluster. AdoMet (red sticks) coordinates a unique Fe of this cluster. The substrate, DTB (black sticks), is located between AdoMet and a 2Fe-2S cluster, which is buried in the core of the barrel and is proposed to be the S donor in the BioB reaction. Fe and S atoms are represented by brown and yellow spheres, respectively.

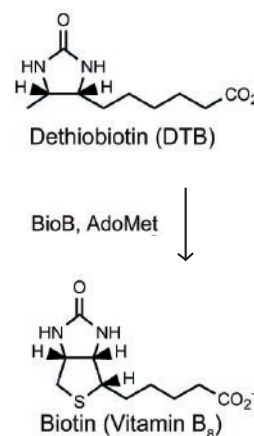


Figure 2. Reaction of BioB

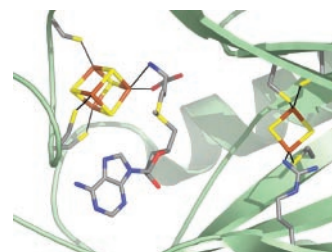


Figure 3. The unique Fe-S clusters of BioB. The 4Fe-4S cluster is shown, coordinated by the CX₂CX₂C motif and by the amino and carboxyl groups of AdoMet. The 2Fe-2S cluster is coordinated by three cysteines and one arginine. All ligands to both clusters are absolutely conserved.